

C-terminal amino acids appeared buried. A search for sequence motifs revealed the presence of one potential N-linked glycosylation site (NAS: aa 15-17), one N-terminal myristoylation site (GKAT(SEQ ID NO:5): aa 1-4), two cAMP-dependent protein kinase phosphorylation sites (KATT(SEQ ID NO:6): aa 2-5; KYKT(SEQ ID NO:7): aa 33-36) and two peroxisomal targeting sequences (GKA: aa 1-3; SKA: aa 54-56). The deduced Ph1 p 6 amino acid sequence displayed identity with a recently submitted Ph1 p 6 sequence (15) and similarities with the N-terminal portions of group 5 grass pollen allergens. However, Ph1 p 6 specific IgE shows little or no crossreactivity with group 5 allergens. A comparison with group 5 grass pollen allergens is given in Vrtala, S., et al., J. Immunol. 1999, 163(10): 5489-5496 (37) (the disclosure of which is incorporated by reference herein). Figure 1A therein shows a multiple sequence alignment, secondary structure and solvent accessibility prediction of Ph1 p 6 variants and group 5 allergens.

B-2
Conclude

Please insert the Sequence Listing enclosed herewith immediately following the abstract.

REMARKS

Enclosed herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a Sequence Listing to be inserted into the specification as indicated above. The Sequence Listing in no way introduces new matter into the specification. The sequences of the Sequence Listing can be found at pages 8 and 10 of the specification.

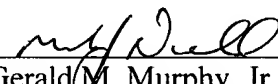
Also submitted herewith in full compliance to 37 C.F.R. §§1.821-1.825 is a disk copy of the Sequence Listing. The disk copy of the Sequence Listing, file "1614-0244P.ST25", is identical to the paper copy, except that it lacks formatting.

Entry of the above amendments is earnestly solicited. An early and favorable first action on the merits is earnestly solicited.

If necessary, the Commissioner is hereby authorized in this, concurrent, and future replies, to charge payment or credit any overpayment to Deposit Account No. 02-2448 for any additional fees required under 37 C.F.R. § 1.16 or under 37 C.F.R. § 1.17; particularly, extension of time fees.

Respectfully submitted,

BIRCH, STEWART, KOLASCH & BIRCH, LLP

By  #36623
Gerald M. Murphy, Jr., #28,977

GMM/MAA/KW
1614-244P

P.O. Box 747
Falls Church, VA 22040-0747
(703) 205-8000

Attachments: -Copy of Notice to Comply with Requirements for Patent Applications
 Containing Nucleotide Sequence and/or Amino Acid Sequence Disclosures
 -Paper Copy of Sequence Listing
 -Disk Copy of Sequence Listing
 -Marked-up version of paragraphs showing changes

Marked-up Version of Paragraphs Showing Changes (additions are underlined and in **bold** - deletions are enclosed by double dashes):

IN THE SPECIFICATION:

Please replace the paragraph on page 8, line 22 with the following amended paragraph:

Construction of hypoallergenic Ph1 p 6 (Phelum pratense) deletion variants.

N-terminal and C-terminal Ph1 p 6 deletion variants were generated to represent aa 1-57 and aa 31-110. cDNAs coding for Ph1 p 6 aa 1-57 and Ph1 p 6 aa 31-110 were obtained by PCR amplification of the Ph1 p 6 cDNA (clone #142) using the following oligonucleotide primers:

For Ph1 p 6 aa 1-57:

5': GGG *AAT TCC* ATA TGG GGA AGG CCA CGA CC 3' (**SEQ ID NO:1**)

5': CGG *GGT ACC* CTA GTG GTG GTG GTG GTG GTG GGG CGC CTT TGA AAC 3' (**SEQ ID NO:2**)

For Ph1 p 6 aa 31-110:

5': GGG *AAT TCC* ATA TGG CAG ACA AGT ATA AG 3' (**SEQ ID NO:3**)

5': CCG *GAA TTC* CTA GTG GTG GTG GTG GTG GTG CGC GCC GGG CTT GAC 3' (**SEQ ID NO:4**)

Eco R I and Kpn I site are printed in italics, Nde I sites and a His-tag, which has been introduced at the C-terminus, are underlined.

Please replace the paragraph beginning on page 9, line 22 and ending on page 10 with the following amended paragraph:

Isolation and characterization of cDNAs coding for isoforms/fragments of Ph1 p 6.

Six cDNA clones (c142, c223, c171, c121, c233, c146), coding for Ph1 p 6 isoforms/fragments were isolated from a timothy grass pollen λ gt11 library with serum IgE from a grass pollen allergic patient. The sequences of the described clones have been deposited in the GenBank database (Accession numbers: Y16955-Y16960). The deduced

amino acid sequences of Ph1 p 6 (clone 142) contained a 28 aa hydrophobic leader peptide. A molecular mass of 11.8 kDa and a pI of 5.5 were calculated for the mature Ph1 p 6 (clone 142) protein which starts with a glycine residue and shows a high content of alanine residues (20.9%). The computer-aided secondary structure analysis on Ph1 p 6 indicates a predominant helical content and the calculation of solvent accessibility predicts that many of the N-terminal amino acids are solvent exposed while most of the C-terminal amino acids appeared buried. A search for sequence motifs revealed the presence of one potential N-linked glycosylation site (NAS: aa 15-17), one N-terminal myristoylation site (GKAT(SEQ ID NO:5): aa 1-4), two cAMP-dependent protein kinase phosphorylation sites (KATT(SEQ ID NO:6): aa 2-5; KYKT(SEQ ID NO:7): aa 33-36) and two peroxisomal targeting sequences (GKA: aa 1-3; SKA: aa 54-56). The deduced Ph1 p 6 amino acid sequence displayed identity with a recently submitted Ph1 p 6 sequence (15) and similarities with the N-terminal portions of group 5 grass pollen allergens. However, Ph1 p 6 specific IgE shows little or no crossreactivity with group 5 allergens. A comparison with group 5 grass pollen allergens is given in Vrtala, S., et al., J. Immunol. 1999, 163(10): 5489-5496 (37) (the disclosure of which is incorporated by reference herein). Figure 1A therein shows a multiple sequence alignment, secondary structure and solvent accessibility prediction of Ph1 p 6 variants and group 5 allergens.